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Detoxification Effects of Wood Vinegar on Aflatoxin B1 in Broiler Chicken

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Abstract: The detoxification effects of wood vinegar were tested in this experiment. A total of 300 healthy Avian broilers at 3 days of age were selected and randomly divided into 5 groups, basal diet, basal diet with 40 μg kg^{-1} Aflatoxin B1 (AFB1), basal diet with 40 μg kg^{-1} AFB1 and 0.5% wood vinegar, basal diet with 40 μg kg^{-1} AFB1 and 1.0% wood vinegar, basal diet with 40 μg kg^{-1} AFB1 and 2.0% wood vinegar. Broilers fed with 40 μg kg^{-1} AFB1 showed significant decrease in marked size and average daily feed intake while feed/gain was increased. In addition, serum total protein and globin were significantly decreased. Wood vinegar could reduce or substantially eliminate the adverse effects from AFB1 on growth performance or tissue damage.

Key words: Wood vinegar, aflatoxin, broiler, growth performance, serum index

INTRODUCTION

Aflatoxins (Aflatoxins, AFT) including Aflatoxin B1 (AFB1), the most widely distributed and chemically stabilized are the secondary metabolites produced by a variety of fungi (Davis *et al*., 1966). Early studies have confirmed that the hazards of AFT to human and animals were associated with its inhibition of protein synthesis, primarily through the interference of RNA and DNA synthesis thus leads to a systemic damage of human and animals (Mishra and Das, 2003; Ellakany *et al*., 2011). It is reported that basal diet contaminated with AFT would cause liver dysfunction, digestive disorder, reduced feed efficiency and decreased immunity (Robens and Richard, 1992; He *et al*., 2013). The functional mechanisms of AFT have been widely researched while few studies focused on the detoxification of AFT. Previous studies have found some new additives could detoxify the disease induced by AFT in animal feed (Ramos and Hernandez, 1997; Huwig *et al*., 2001; Shi *et al*., 2009). Thus, study and exploit a new and safety adsorbent is essential for sustainable development of animal husbandry.

Wood vinegar is a liquid product obtained from the process of production of charcoal which is an ark red-brown liquid contained many components, water, organic acids, phenols, ketones, alcohols, etc. (Loo *et al*., 2007). It can be used for soil disinfection, sterilization and disease prevention as well as feed additives and agricultural chemicals when under the condition of pH 2.5-3.0 and contained 2.0-6.1% acetic acid (Samanya and Yamaguchi, 2001; Mekbungwan *et al*., 2004; Paraud *et al*., 2011; Pangnakorn *et al*., 2011; Yamauchi *et al*., 2013). In this experiment, three yellow chickens were selected for the detection of detoxification effects of wood vinegar as feed additive when basal diet contaminated with AFB1. Through monitoring the growth performance, change of serum biochemical indices and toxin residues, comprehensively evaluate the application of wood vinegar as new feed additive in animal production.

MATERIALS AND METHODS

Materials: Wood vinegar was obtained from Northeast Forestry University, Haerbin, China. AFTB1 was purchased from Fermentek (Fermentek, Inc.) with a purity above 98%.

Animals and diets: A total of 300, 1 day old Avian broiler chickens were individually weighed, wing banded and randomly distributed to 5 treatment groups by equal sex ratio. Chickens were fed for 42 days under standard management conditions with feed and water available *ad libitum*. All diets contained adequate levels of nutrients as recommended by the National research council. The composition and nutrient content of the basal diet are presented in Table 1. Experimental diets for each treatment were as follows: group A: basal diet as control treatment, group B: basal diet with 40 μg kg^{-1} AFBI, group C: basal diet with 40 μg kg^{-1} AFBI and 0.5% wood vinegar, group D: basal diet with 40 μg kg^{-1} AFBI and 1.0% wood vinegar and group E: basal diet with 40 μg kg^{-1} AFBI and 2.0% wood vinegar (Table 2).

Measurements: Body weight and feed consumption were measured weekly and mortality was recorded. At the end of the trial, chicks were killed by cervical dislocation and necropsied. All livers in each group were removed and

weighed. Serum concentrations of Total Protein (TP), Albumin (ALB), Globin (GLOB) and activities of Glutamic-Pyruvic Transaminase (GPT) and Glutamicoxalacetic Transaminase (GOT) were determined on a biochemical autoanalyzer according to the manufacturer's recommended procedure (Beckman Instruments, Inc.)

AFTB1 from the liver and serum were determined as previously described (Shi *et al*., 2009). A sample of 0.2-0.5 g liver (0.2-0.5 mL serum) was cut into small pieces and blended for 2 min at high speed with 20 mL of dichloromethane and 2 g of diatomaceous earth. After washing further with 15 mL dichloromethane, the mixture was filtered and pressed to release maximum amount of filtrate. The filtrates were combined and evaporated in a rotary evaporator at 40°C. The residue was dissolved in 1 mL methanol methanol, 30 mL water and 50 mL n-hexane and transferred to a separating funnel. The water phase (lower phase) and the hexane phase were then washed twice with 10 mL water and the water phase also collected. Subsequently both water phases were homogenized and then applied to an immunoaffinity column. The toxin was eluted from the column using 1 mL methanol and then analyzed using HPLC.

Table 1: Composition and nutrient levels of the basal diet (air-dry basis)

Ingredients (%)	0-3 weeks	4-6 weeks
Composition		
Corn	57.00	58.00
Flour	5.00	8.00
Soybean meal	26.00	21.00
Rapeseed meal	2.00	2.70
Cottonseed meal	2.00	3.00
Fishmeal	2.50	1.00
Mixed oil	1.00	1.10
$CaHPO_4$	1.20	1.20
Limestone	0.30	0.30
NaCl	2.00	2.70
Premix*	1.00	1.00
Nutrient level		
ME	12.04	12.26
CP	20.34	18.39
Ca	0.95	0.91
TP	0.68	0.67
Lys	1.05	0.89
Met	0.34	0.30

*The premix provided the following per kg of diet, Fe 80 mg, Cu 8 mg, Zn 60 mg, Mn 80 mg, VA 5000 IU, VD3 1000 IU, VE 10 IU, riboflavin 3.6 mg, niacin 35 mg; pantothenic acid 10 mg, VB12 0.01 mg, biotin 0.15 mg; choline 800 mg

Table 2: Composition of diet in each group

Groups	Diet composition
A	Basal diet
B	Basal diet+40 μg kg^{-1} AFBI
C	Basal diet+40 μg kg^{-1} AFBI+0.5% wood vinegar
D	Basal diet+40 μg kg^{-1} AFBI+1.0% wood vinegar
E	Basal diet+40 μg kg^{-1} AFBI+2.0% wood vinegar

Statistical analysis: All data were subjected to statistical analysis using the General Linear Models procedure of the Statistical Analysis System 9.3. The significance of the differences among the treatment groups with variable means was determined by one-way ANOVA Method. Results are presented as means±SEM of measurements. All statements of significance were based on $p<0.05$.

RESULTS

Growth performance of broiler chickens fed with wood vinegar: The effects of AFTB1 and wood vinegar on growth performance are shown in Table 3. Broiler chickens fed with basal diet with 40 μg kg^{-1} AFB1 showed an obvious negative effect on chicken performance (Table 3). There was no significant difference in feed intake in each group ($p>0.05$). A significant decrease in market size (12.1%) and 10.9% increase in F/G was observed in group B while compared with group A. In group C-E, addition of 0.5, 1.0 and 2.0% vinegar, respectively in basal diet contained with 40 μg kg^{-1} AFB1, an obvious increase in market size and decrease in F/G compared with group B was observed ($p<0.05$) while there was no significant difference in market size and F/G compared with group A. these suggested that wood vinegar could alleviate the loss of production performance from AFB1.

Analysis of the AFB1 residue in serum and liver: Broiler chickens fed with diet contaminated with 40 μg kg^{-1} AFB1 showed a high serum AFB1 residue with 0.065±0.011 ng g^{-1} which was 5 times higher than that of group A ($p<0.05$). There was no significant difference in serum AFB1 residue in groups fed with different content of wood vinegar compared to group B however, a numerically decease with 20.0, 26.2 and 35.4%, respectively was observed compared to group B, suggested a dose dependent decrease in AFB1 residue in serum.

Liver AFB1 residue in group B was 10.45±1.45 ng g^{-1} which was significantly higher than that of group A ($p<0.05$). Liver AFB1 residue in group C-E was decreased with increased addition of wood vinegar with 21.7, 32.5

Table 3: Effects of adding wood vinegar to basal diet contaminated with 40 μg kg^{-1} AFB1 on growth performance of broiler chickens

Groups	Average daily feed intake (g)	Market size (g)	Feed/Gain (F/G)
A	65.55±7.22	1302.55±40.42^a	2.21±0.10^a
B	64.52±5.85	1145.25±42.38^b	2.45±0.11^b
C	68.55±7.23	1338.82±38.55^a	2.19±0.09^a
D	68.23±5.52	1342.32±40.65^a	2.20±0.09^a
E	66.48±6.72	1325.65±36.58^a	2.18±0.12^a

Values within a row with the different superscript letters differ significantly ($p<0.05$)

Table 4: AFB1 residue in serum and liver of broiler chickens

Groups	AFBI residue in serum (ng g^{-1})	AFBI residue in liver (ng g^{-1})
A	0.012±0.006^{a}	3.32±0.28^{a}
B	0.065±0.011^{b}	10.45±1.45^{b}
C	0.052±0.016^{b}	8.22±0.95^{bc}
D	0.048±0.017^{b}	7.05±1.25^{bc}
E	0.042±0.021^{b}	6.28±1.22^{c}

Table 5: Effects of wood vinegar addition on serum enzyme activity and protein content of broiler chickens

Groups	GOT	GPT	TP	ALB	GLB
A	185.46±14.23	1.76±0.11	50.60±2.89^{a}	20.24±1.52	31.12±1.85^{a}
B	192.25±12.35	2.18±0.22	41.25±2.55^{b}	18.42±1.05	24.88±1.77^{b}
C	188.48±10.26	2.01±0.18	43.22±2.12^{b}	19.58±1.55	26.45±1.56^{ab}
D	194.82±12.65	1.88±0.10	44.52±1.68^{b}	20.33±1.85	27.52±2.65^{ab}
E	196.55±11.43	1.82±0.15	47.21±2.05^{ab}	20.15±1.65	29.55±1.88^{ab}

and 39.1% decrease compared to group B and the liver AFB1 residue in group E was significantly lower than that of group B ($p<0.05$).

Addition with different ratio of wood vinegar could decrease the AFB1 residue in serum and liver in a certain level. Addition with 2.0% wood vinegar could significantly decrease liver AFB1 residue when broiler chickens fed with basal diet contained 40 μg kg^{-1} AFB1 (Table 4).

Effect of wood vinegar on change of serum chemicals of broiler chicken: There were no significant different in aspartate aminotransferase activity, alanine aminotransferase activity and albumin level in each group ($p>0.05$). While the serum total protein in broiler chickens fed with diet contained with 40 μg kg^{-1} AFB1 was significantly decreased ($p<0.05$) and wood vinegar addition could effectively restore the total protein content in a dose dependent level. AFB1 in basal diet caused the globulin decreased from 31.12±1.85-24.88±1.77 U mL^{-1} and addition with wood vinegar could increase the globulin in a certain degree however the globulin level in group C-E only showed a numerically increase with no significant difference compared to group A or B. In conclusion, basal diet contaminated with AFB1 could cause the decrease of serum total protein, albumin and globulin content while wood vinegar addition could increase these indexes in a certain degree (Table 5).

DISCUSSION

Aflatoxins are a group of highly toxic substances, the toxicity highly associate with the dose, duration, species, rearing condition, diet, nutrient, etc. during animal production; there is few acute poisoning accident but mainly the chronic poisoning (Robens and Richard, 1992; Wild and Turner, 2002). In poultry production, the chronic poisoning would cause growth inhibition including reducing body weight gain, decreased feed conversion ratio (Shi *et al*., 2009; Cai *et al*., 2011; He *et al*., 2013). In this experiment, broiler chickens fed with diet contained AFB1 showed decreased body weight gain and increased F/G which was in accordance with other reports. The addition of wood vinegar, the production performance was significantly improved which suggest that wood vinegar alleviated the negative effects caused by AFB1.

The major metabolism of AFTB1 occurred in liver through hydroxylation, demethylation and epoxidation (Swenson *et al*., 1977; Wilson and Jones, 1983). Thus, the metabolism of AFTB1 in liver is in fact, the detoxification process that would cause serious damage in liver. In this experiment, after intake of diet contained AFTB1, the AFTB1 residue was detected in serum and liver in broiler chickens. While addition with the non-nutritional additive of wood vinegar, the accumulation of AFTB1 showed a linear decrease in serum and liver.

Aflatoxin could change the activity of many kinds of enzymes. With liver damage, the enzyme, secreted from liver, activity would change. Therefore, the enzyme activity can be used to access the damage animals suffered from (Kew, 2003). It is reported that AFT can be combined to nucleic acid and proteins that caused the change of the activity of DNA template or the inactivation of enzymes which participated in the synthesis of DNA or RNA thus lead to the disruption of protein synthesis (Madrigal-Santillan *et al*., 2007). AFB1 can interfere the ribosome binding sites on endoplasmic reticulum and interfere the ribosome cycle and inhibit the release of newly synthesized protein (Madden *et al*., 2002). Low serum protein content has been taken as the general effect of poisoned by aflatoxin (Huwig *et al*., 2001). In this experiment, broiler chickens fed with diet contained AFTB1 showed significant decrease of total protein, albumin and globulin, suggest that AFTB1 might caused liver cell damage, the decline of immunity which was highly in accordance with reports mentioned above. Wood vinegar showed obvious improving effect in such serum protein content.

CONCLUSION

Broiler chickens fed with diet contained AFB1 caused declined body weight, increased F/G while addition with wood vinegar improved body weight gain and reduced feed consumption. AFB1 residue was detected in serum and liver in broiler chickens fed with diet contained AFB1 and the accumulation of AFB1 in serum and liver showed numerically decreased with the increased dose of wood vinegar. Broiler chickens fed with diet contained AFB1 induced decreased content of serum total protein and globulin, the addition of wood vinegar alleviated this toxicity.

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